Proc. Natl. Acad. Sci. USA
Vol. 73, No. 10, pp. 3734–3738, October 1976
Physiology

An oscillatory neuronal circuit generating a locomotory rhythm

(medicinal leech/swimming/interganglionic coordination/electronic analog model)

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Communicated by Donald A. Glaser, August 9, 1976

ABSTRACT **A quartet of interconnected interneurons whose periodic activity appears to generate the traveling body wave of the swimming leech has been identified on each side of segmental ganglia of the ventral nerve cord of *Hirudo medicinalis*. Theoretical analysis and electronic analog models of the identified intra- and interganglionic synaptic connections of the segmentally iterated interneurons showed that they form an oscillatory network with cycle period and intra- and intersegmental phase relations appropriate for the swimming movement.**

Leech swimming

Leeches swim by undulating their extended and flattened body in the dorsoventral direction, to form a wave that travels backwards along the animal. The moving troughs and crests of this body wave are produced by a metachronal rhythm of antiphasic contraction-distension cycles of the dorsal and ventral longitudinal muscles in the body wall of each of the 21 somatic segments. The period of this rhythm varies from about 400 to 2000 msec. Previous studies (1–3) have shown that the swimming rhythm in the medicinal leech, *Hirudo medicinalis*, is controlled by an ensemble of bilateral pairs of excitatory and inhibitory motor neurons present in each of the segmental ganglia of the leech ventral nerve cord. This neuronal ensemble includes excitors (cells 3, 5, 7, and 107) and inhibitors (cells 1 and 102) of the dorsal longitudinal muscles, as well as excitors (cells 4, 8, and 108) and an inhibitor (cell 2) of the ventral longitudinal muscles (Fig. 1A). Central inhibitory synaptic connections link the inhibitors with the excitors. During swimming the membrane potential of these motor neurons oscillates between a depolarized and a hyperpolarized state, with an impulse burst arising during the depolarized state.

The phases of the rhythms of motor neuron activity, as determined by the timing of the middle impulse of each impulse burst and with the standard phase angle 0° arbitrarily assigned to the rhythm of cell 3, are approximately 90° for cell 1, 180° for cells 4 and 102, and 270° for cell 2 (Fig. 2A). Furthermore, in accord with the rearward travel of the body wave, the impulse burst phase of each of these motor neurons leads that of its serial homolog in the next posterior segmental ganglion. Execution of the swimming movement can, therefore, be accounted for by the activity pattern of this ensemble of identified motor neurons. The motor neurons are not, however, the source of their own activity pattern (2). Instead, the swimming rhythm must be imposed on them by other, oscillatory neural elements.

Kristan and Calabrese (4) discovered recently that the motor neurons of an *isolated* leech ventral nerve cord can exhibit sustained episodes of the swimming rhythm. The motor neurons of a single, isolated ventral cord ganglion do not, however, manifest that rhythm; a chain of at least six to eight segmental ganglia appears to be required for generation of the rhythm. Thus, the central nervous system of the leech contains an oscillator whose intra- and interganglionic connections can drive the motor neurons to produce periodic impulse bursts in the absence of any peripheral afference. This report presents the identification of a set of interconnected interneurons which appears to make up the central swimming oscillator that drives the motor neurons. A brief account is also provided of the proposed mechanism by which the interneuronal network produces the coordinated activity rhythm of its elements*.

Identification of the oscillator interneurons

A search was carried out in preparations of isolated ventral nerve cords of *H. medicinalis* for neurons that manifest the following properties diagnostic of components of the central oscillator: the membrane potential oscillates with a period matching that of the swimming rhythm, and passage of current into the neuron resets the phase of the motor neuron activity cycles. This search led to four bilateral pairs of neurons, namely, cells 123, 28, 33, and 27, all having very small cell bodies located on the dorsal aspect of the segmental ganglia (Fig. 1A). Passage of depolarizing current into any one of these four rhythmically active cells resets not only the phase of its own activity cycle, but also the phase of the impulse burst rhythm of the dorsal excitor, cell 3, in other ganglia of the isolated nerve cord (Fig. 1C). The activity cycles of the oscillator cells 123, 28, 33, and 27, as determined from records such as those shown in Fig. 1C, occur in a phase progression corresponding to phase angles of about 0°, 90°, 180°, and 270°, respectively (Fig. 2A).

Anatomical as well as electrophysiological evidence suggests that the oscillator cells are intersegmental interneurons. First, upon specific staining of cell 28 and cell 33 by intracellular injection of horseradish peroxidase (5), both cells could be seen to send an axon into the anterior connective, and neither cell could be seen to send an axon into the segmental nerve roots of its ganglion (Fig. 1B). Second, impulses arising in cell 27, cell 28, or cell 33 could be recorded in the ventral cord connective over a distance of at least five segments to the front, and impulses arising in cell 123 could be similarly recorded over a distance of at least two segments to the rear. No trace of the impulses of any of these four cells could be found in the segmental nerves. Thus, cells 27, 28, and 33, whose cell bodies lie in the posterior packet of the ganglion, project to more anterior ganglia, and cell 123, whose cell body lies in the anterior packet, projects to more posterior ganglia.

The interneuronal network

The oscillator network contains both *intra*ganglionic and *inter*ganglionic connections of the interneuron quartet. The connections identified thus far are summarized in Fig. 2B. In

* The methods of dissecting and mounting preparations, of taking intracellular recordings from nerve cell bodies by means of glass capillary microelectrodes and extracellular recordings from segmental nerves by means of glass-tipped suction electrodes, of passing current into nerve cells, and of numbering segments and designating the components of the segmental nerve system were those previously described (1, 2, 4).

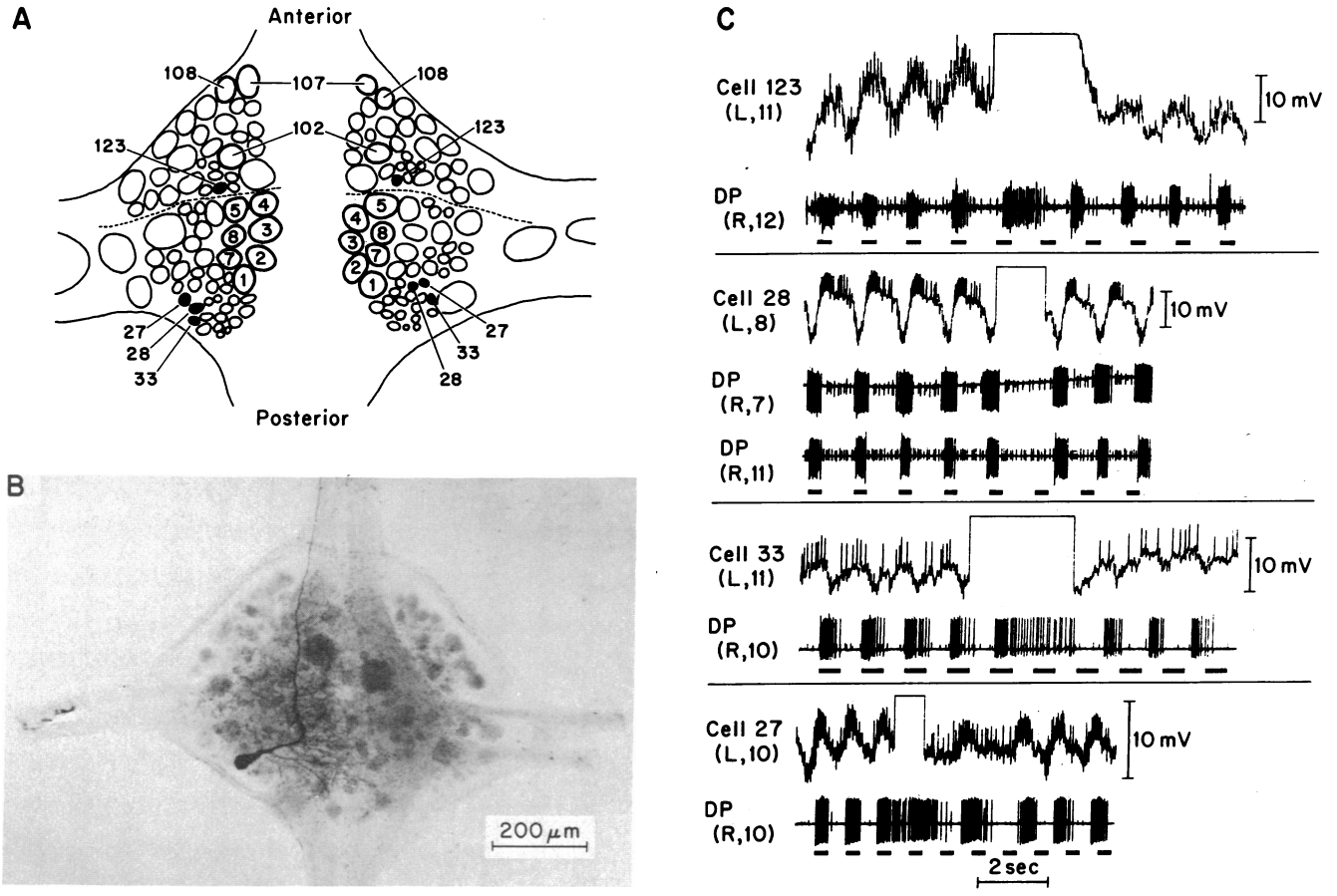

FIG. 1. (Panel A) Dorsal aspect of a segmental ganglion of the ventral nerve cord of the medicinal leech, *H. medicinalis*, showing the cell bodies of identified motor neurons (heavy outline) and of interneurons (solid black) taking part in the generation of the swimming rhythm. The cells are numbered according to the system of Ort, Kristan, and Stent (2). (Panel B) Anatomy of an oscillator interneuron. Photograph of the dorsal aspect of a ganglion in which the left cell 33 was stained by intracellular injection of horseradish peroxidase. (Panel C) Simultaneous intracellular microelectrode recordings taken from an interneuron (cell 123, cell 28, cell 33, or cell 27) of midbody ganglia and extracellular suction electrode recordings taken from the dorsal branch of the posterior segmental nerve (DP), in nearby segments. The large amplitude spikes in the DP records represent impulses of the dorsal excitor, cell 3. In this and the following electrophysiological records presented here, the letters R or L following in parentheses the designation of a cell or segmental nerve indicate right or left side, respectively, and the number indicates the segment from which the recording was taken. A sharp upward deflection of the intracellular traces marks passage into the interneuron of a pulse of depolarizing current of no more than 5×10^{-9} amp. The bars drawn under the DP records indicate the times of occurrence of impulse bursts from cell 3 to be expected if passage of current into the interneuron had *not* reset the phase of the swimming rhythm.

this diagram the four interneurons of two ganglia—one more anterior and the other more posterior—have been placed at the corners of a square, so that their activity phases progress clockwise. *Intra*ganglionically, each interneuron makes inhibitory connections with the cells that lead it by phase angles of 90° and 180° in the activity rhythm [except for one interneuron (cell 123), which connects only with the cell that leads it by 90°]. *Inter*ganglionically, the three interneurons (cells 28, 33, and 27) whose axons project into the anterior connective make inhibitory connections in more anterior ganglia with the serial homologs of one or both of the cells with which they also connect in their own ganglion. The one interneuron (cell 123) whose axon projects into the posterior connective, however, makes inhibitory connections in more posterior ganglia with the serial homologs of the cell that in its own ganglion *follows* it by a phase angle of 90°. Hence, in contrast to the frontward interganglionic connections, the rearward interganglionic connection has no intraganglionic homolog.

This network was identified by pairwise intracellular recordings taken from interneurons located either in the same ganglion or in different ganglia. On the basis of these recordings, two cells were inferred to be connected if passage of current into one cell produced a change in membrane potential or impulse frequency in the other cell. This test does not show whether or not the inferred connection is monosynaptic. Sample records that identified intraganglionic connections are presented in Fig. 3A. In this figure, the first pair of traces shows that passage of depolarizing current into cell 28 hyperpolarized cell 123, whereas hyperpolarizing current slightly depolarized cell 123. Hence, it appears that cell 28 connects to cell 123 via an inhibitory synaptic link. Records not presented here showed that similar inhibitory connections exist from cell 123 to cell 27 and from cell 27 to cell 33. An inhibitory connection from cell 33 to cell 123 was inferred to exist from the finding that cell 123 manifests inhibition concomitantly with post-inhibitory rebound activity in cell 33. The second pair of traces of Fig. 3A shows that passage of depolarizing current into cell 33, as well as post-inhibitory rebound of cell 33, hyperpolarized cell 28. Passage of depolarizing current into cell 28 had no observable effect on cell 33, whereas hyperpolarization of cell 28 hyperpolarized cell 33. These findings indicate that cells 33 and 28 are connected not only via an inhibitory synaptic link from cell

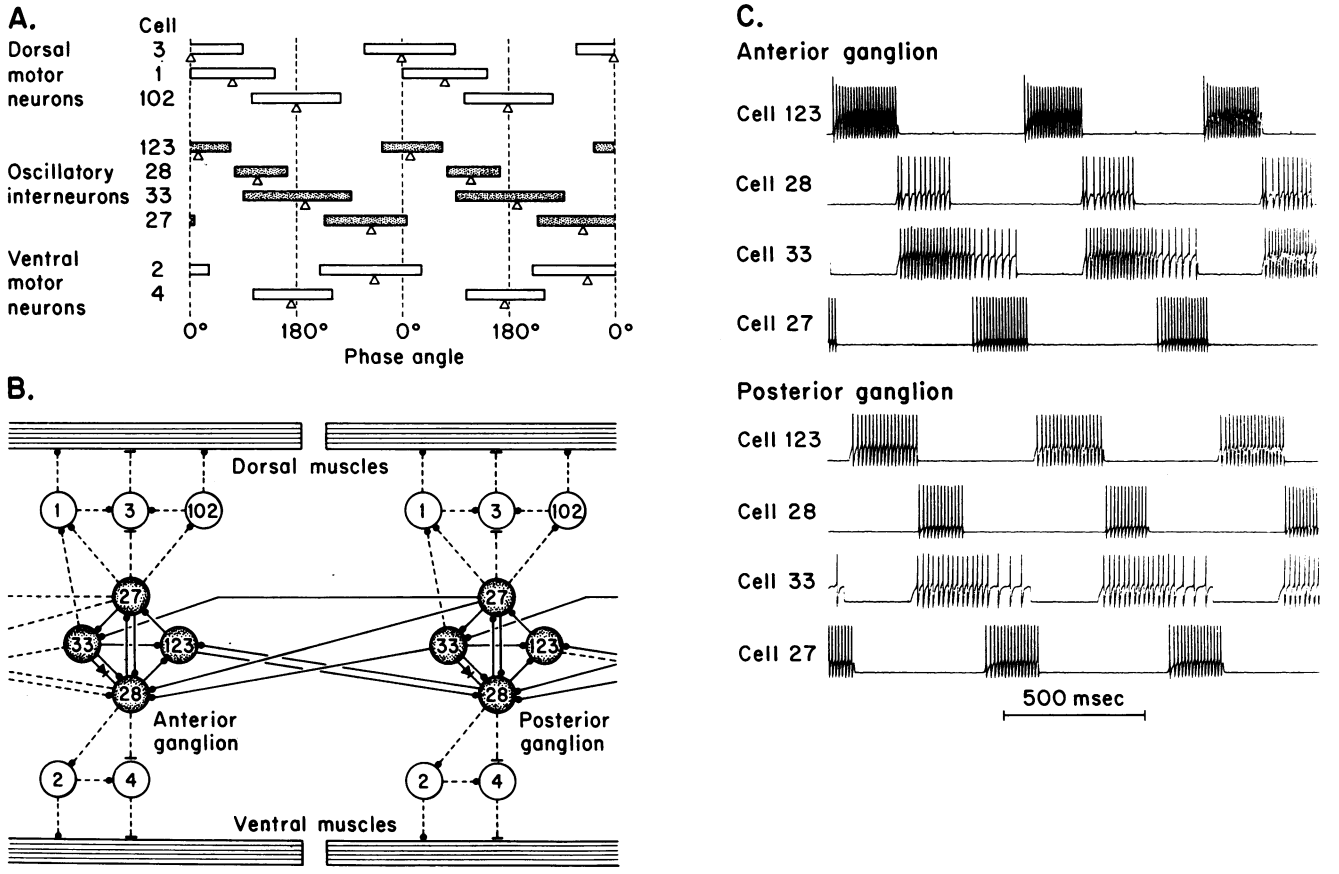

FIG. 2. (Panel A) Phase diagram of the swimming activity cycles of motor neurons and interneurons. Each bar indicates the duration of the impulse burst of the cell; the triangle under the bar points to the burst midpoint, or middle spike. The burst midpoint of cell 3 has been arbitrarily assigned the phase angle 0°. (Panel B) Summary circuit diagram of identified synaptic connections between interneurons, motor neurons, and longitudinal muscles responsible for the swimming rhythm. The dashed lines represent connections that were not included in the electronic analog circuit. Meaning of symbols: T joint = excitatory synapse; filled circle = inhibitory synapse; diode = rectifying electrical junction. (Panel C) Impulse bursts generated by an electronic analog model of the circuit formed by the connections shown in solid lines in panel B. Each trace is the output of the "neuromime" element corresponding to one of the eight oscillatory interneurons. The model envisaged an anterior and a posterior ganglion separated by four segments, and hence a total interganglionic impulse conduction time of 80 msec. Lack of a sufficient number of neuromime elements prevented modeling of the *inter*ganglionic inhibitory inputs shown in panel B to reach the interneurons of the posterior ganglion from the rear. However, these inputs were simulated in the analog model by introducing into the posterior ganglion additional *intra*ganglionic inhibitory connections from cell 27 to cells 33 and 28, from cell 33 to cell 28, and from cell 28 to cell 123. These additional connections incorporated a transmission delay of 160 msec, in order to mimic both a phase lag of 80 msec and a conduction time of 80 msec appropriate for inputs reaching the posterior ganglion from a ganglion four segments to its rear.

33 to cell 28, but also via a rectifying electrical junction, which permits passage of hyperpolarizing but not of depolarizing current from cell 28 to cell 33. The third pair of traces of Fig. 3A shows that passage of depolarizing current into either member of the cell 27-cell 28 pair hyperpolarized the other member, leading to the inference that cell 27 and cell 28 are connected via reciprocal inhibitory synaptic links. In addition to the intraganglionic connections shown in Fig. 2B, two of the interneurons (cells 28 and 33) have been found to be linked by an electrical junction to their contralateral homologs; thus the bilateral pair of ganglionic oscillators is coupled.

Sample records that identified some of the interganglionic connections are presented in Fig. 3B. In this figure the first pair of traces shows that passage of depolarizing current into cell 28 hyperpolarizes cell 123 of a more anterior ganglion and that passage of depolarizing current into cell 123 hyperpolarizes cell 28 of the next posterior ganglion. Cells 123 and 28 of different ganglia are thus reciprocally connected via inhibitory synaptic links in such a manner that cell 123 makes an inhibitory contact with cell 28 in more posterior ganglia and cell 28 makes an inhibitory contact with cell 123 in more anterior ganglia. Thus, the intraganglionic inhibitory effect of cell 28 on cell 123 is repeated interganglionically. The second pair of traces of Fig. 3B shows that passage of depolarizing current into cell 27 hyperpolarized cell 28 of the next anterior ganglion. Hence, cell 27 repeats its intraganglionic inhibitory contacts with cell 28 interganglionically, by contacting cell 28 in more anterior ganglia. Other records not presented here show that cells 27 and 33 make inhibitory contact with cells 33 and 28, respectively, of more anterior ganglia.

Output connections of the oscillator network

The oscillator network imposes the swimming rhythm on the motor neurons via excitatory and inhibitory connections. Sample records that identified these connections are presented in Fig. 3C. In this figure, the first set of traces shows that impulses in cell 33 are followed with constant delays of less than 5 msec and about 40 msec by an inhibitory synaptic potential in the ipsilateral dorsal inhibitor, cell 1, in the same ganglion and in a ganglion two segments more anterior, respectively. The constant delay of 40 msec observed here indicates an impulse conduction time of about 20 msec per segment in the inter-

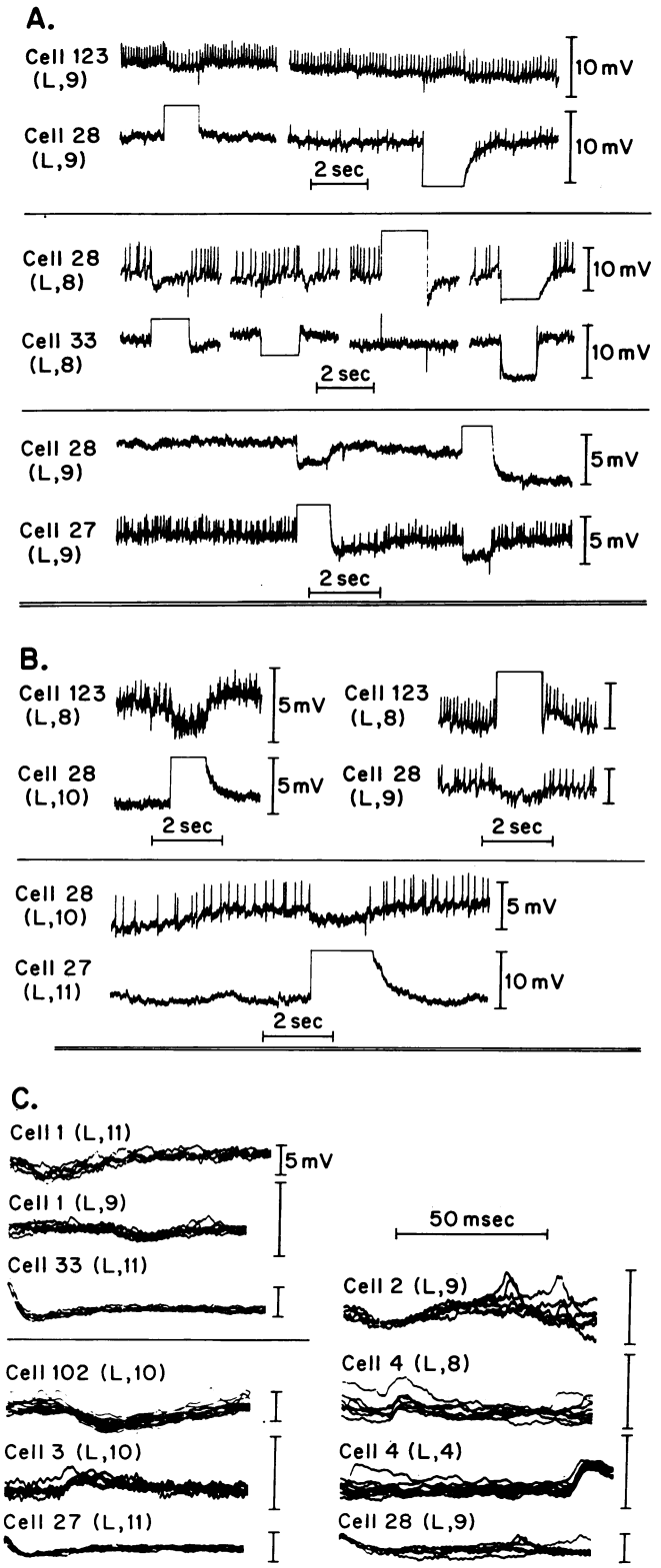

FIG. 3. Intra- and interganglionic connections of the interneurons. (Panels A and B) Pairwise intracellular recordings taken from oscillator interneurons within the same ganglion (panel A) or different ganglia (panel B). Sharp upward or downward deflections of the traces mark intracellular passage of depolarizing or hyperpolarizing current, respectively. (Panel C) Composite superimposed oscilloscope sweeps of simultaneous intracellular recordings from an interneuron and motor neurons in different ganglia, triggered by impulses in the interneuron. Unless indicated otherwise, vertical calibration marks represent 5 mV.

segmental axon of cell 33. The lower left set of traces of Fig. 3C shows that impulses in cell 27 are followed with constant delay by an excitatory synaptic potential in the ipsilateral dorsal excitor, cell 3, and by an inhibitory synaptic potential in the dorsal inhibitor cell 102, of the next anterior ganglion. Records not presented here show that impulses in cell 27 are also followed by an inhibitory synaptic potential in the ipsilateral dorsal inhibitor, cell 1, in the next anterior ganglion. The remaining set of traces of Fig. 3C shows that impulses in cell 28 are followed with constant delays by an inhibitory synaptic potential in the ipsilateral ventral inhibitor, cell 2, of the same ganglion and by an excitatory synaptic potential in the ipsilateral ventral excitor, cell 4, in the next anterior ganglion and a ganglion five segments to the front. Records not presented here show that cell 28 also provides excitatory input to the ipsilateral cell 102 of the next anterior ganglion, and that cell 123 provides excitatory input to the ipsilateral cell 3 of the next posterior ganglion. A diagrammatic summary of these connections between interneurons and motor neurons, as well as of the previously established connections between inhibitors and excitors, and between motor neurons and longitudinal muscles, is included in Fig. 2B. (For the sake of clarity, the excitatory links from cell 123 to cell 3 and from cell 28 to cell 102 have been omitted.) In view of these excitatory and inhibitory connections, the oscillatory activity pattern of the interneurons shown in the phase diagram of Fig. 2A can account for the major features of the activity pattern of the motor neurons during swimming.

Mechanism of the oscillation

The identified intraganglionic connections of Fig. 2B between the four interneurons and the phase progression of their activity cycles correspond almost exactly to a hypothetical oscillatory circuit of an interneuron quartet first proposed by Székely to account for the activity rhythm of spinal cord motor neurons in control of amphibian locomotion (6). The only difference between the intraganglionic connections diagrammed in Fig. 2B and the Székely oscillator network is that the latter includes a connection corresponding to an inhibitory link from cell 123 to cell 33 and lacks a connection corresponding to the rectifying electrical junction between cells 33 and 28. By means of both theoretical arguments and electronic analog models, Kling and Székely (7) could show that sequential disinhibition around the inhibitory loop would produce a progression of rhythmic impulse bursts in four tonically excited interneurons, without the need to invoke either post-inhibitory rebound or synaptic fatigue. The period of this oscillator is equal to the sum of the recovery delays between the start of disinhibition of each of the four interneurons and the onset of its impulse burst. Measurement of these recovery delays for the leech oscillator cells from data such as those shown in Fig. 3A indicates that after termination of a depolarizing current pulse passed into the presynaptic cell, each of the four interneurons recovers from inhibition within 50 msec. Hence, if only *intra*ganglionic connections entered into the operation of the oscillator, the swim period under the Székely model could not exceed the unrealistically low value of 200 msec. However, this difficulty disappears as soon as the identified *inter*ganglionic connections and their interganglionic impulse conduction delays of about 20 msec per segment are also taken into account. First, the inhibitory link from cell 123 to cell 28 in more posterior ganglia assures that the swim cycle of each ganglion must lag behind that of its immediately preceding ganglion by at least 20 msec. As a consequence of this progressive rearward delay of the swim cycles, there occurs a phase divergence of the inhibitory input supplied to cells 123, 28, and 33 of a given ganglion from cells

27, 28, and 33 of more posterior ganglia. Compared to the inhibitory input which cell 123, or cell 28, or cell 33 receives from an interneuron of its own ganglion, the input from the serially homologous interneuron n segments to the rear will be delayed, and hence the hyperpolarized inactive phase will be extended, by $2n \times 20$ msec. Although the tests of Fig. 3B established frontward interganglionic connections between interneurons over a distance of only two segments, the finding that these interneuronal axons form iterated connections with motor neurons for at least five segments makes it seem likely that the frontward connections between interneurons also extend for at least five segments. Therefore, n is likely to have a value no less than five. Accordingly, the hyperpolarized phase of each of the interneurons receiving inhibition from posterior ganglia is extended by about 200 msec, thus lengthening the oscillator cycle by several hundred msec to realistically long periods.

In order to validate these theoretical considerations, an electronic analog model of the eight interneurons and their intra- and interganglionic connections shown in Fig. 2B was constructed. The model included interganglionic impulse conduction delays appropriate for two ganglia separated by four segments†. The output of this analog model is presented in Fig. 2C. As can be seen, the model oscillator runs with a realistic swim period of about 700 msec, reproduces in good approximation the physiological interneuronal phase relations shown in Fig. 2A, and gives rise to an appropriate phase delay between anterior and posterior ganglia of about 100 msec. Thus, both the experimental evidence and the modeling results make it appear that the network of interneurons diagrammed in Fig. 2B gives rise to the leech swimming rhythm.

† The electronic analog model consisted of eight interconnected "neuromime" elements (8). Each neuromime element was excited tonically to generate impulse activity at a frequency of about 100 Hz. Interganglionic impulse conduction delays were modeled by means of a shift register. A detailed description of the analog circuit and of its output will be published elsewhere.

We are indebted to W. B. Kristan, Jr. and R. L. Calabrese for making available to us their data prior to publication and to Donald Kennedy for providing us with neuromime elements. This work was supported by Research Grants GM 17866 and NS 12818 from the National Institutes of Health and GB 31933X from the National Science Foundation; and by NIH Postdoctoral Fellowship NS 05144.

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